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Effects of H_2-Receptor Antagonists Cimetidine, Ranitidine, and ICI 125,211 on Histamine-Stimulated Adenylate Cyclase Activity in Guinea Pig Gastric Mucosa

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SUMMARY

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Histamine stimulation of adenylate cyclase activity in broken cells isolated from guinea pig gastric mucosa was competitively inhibited by the thiazole derivative ICI 125,211 and the furane derivative ranitidine, with pA_2 of 7.62 ($K_i = 2.39 \times 10^{-8}$ M) and pA_2 of 6.90 ($K_i = 1.25 \times 10^{-7}$ M), respectively, i.e., potencies 19 times and 4 times superior to that of the classical H_2-receptor antagonist cimetidine ($pA_2 = 6.34$; $K_i = 4.57 \times 10^{-7}$ M). These results are consistent with the reported pharmacological potencies of the antagonists on guinea pig right atrium and on gastric acid secretion *in vivo*. Thus, the requirement of an imidazole ring in the structure of H_2-receptor antagonists can be definitely ruled out.

INTRODUCTION

Since the earlier work by Perrier and Griessen (1), the effects of histamine on gastric mucosal cells have been well documented, especially in guinea pig, in terms of adenylate cyclase stimulation (2, 3), cyclic AMP production (4, 5), and cyclic AMP-dependent protein kinase activation (6, 7). These effects have been suggested to reflect intracellular events relevant to histamine stimulation of gastric acid secretion through specific "H_2" receptors. So far, however, biochemical approaches to the characterization of gastric histamine receptor have been restricted to studies involving a unique class of H_2 antagonists, i.e., the imidazole analogues burimamide (8), metiamide (9), and cimetidine (10). The recent discovery of a new series of H_2 antagonists with different molecular structure offered the possibility of obtaining new information on the structure of the H_2 receptor. In this study, the effect of cimetidine on adenylate cyclase activity of guinea pig gastric cells was compared with the effects of ranitidine (11) and ICI 125,211 (12), two recently reported H_2 antagonists bearing, respectively, a furane ring and a thiazole ring.

MATERIALS AND METHODS

Fed male guinea pigs weighing between 300 and 400 g were killed by a blow on the neck. The stomachs were excised, the fundic mucosa was scraped, and gastric epithelial cells were isolated as previously described (4, 13). The effect of histamine and its antagonists was examined in broken-cell preparations (10-sec sonication at 6 μm, 20 kHz). Adenylate cyclase activity was measured according to the method of Krishna *et al.* (14) using [α-^{32}P]ATP as substrate (0.2 mM, specific activity about 20 mCi/mmole) and with the modification reported by Lewin *et al.* (13).

Quantitative analysis of the data was made according to the classical Michaelis-Menten description of agonist- or antagonist-receptor interaction in the absence of cooperative effect, i.e., $RH = H \cdot (Rt - RI)/(K + H)$ and $RI = I \cdot (Rt - RH)/(K_i + I)$, where RH and RI represent, respectively, receptors occupied at equilibrium by agonist and antagonist at concentrations H and I; K and K_i represent agonist- and antagonist-receptor dissociation constants; and Rt represents total receptor concentration. Assuming that adenylate cyclase activity (E) is proportional to RH, the above equations lead to the following: $E = E_{max} \cdot H \cdot K_i/(K \cdot K_i + I \cdot K + H \cdot K_i)$ (Eq. 1). In the absence of antagonist, Eq. 1 simply reduces to $E = E_{max} \cdot H/(H + K)$, or $\log (E_{max}/E - 1) = \log K - \log H$ (Eq. 2). In the presence of antagonist, Eq. 1 can be rewritten as: $K_i = a/(1 - a) \cdot K \cdot I/(K + H)$ or $\log [a/(1 - a)/] = \log K_i - \log I + \log (1 + H/K)$ (Eq. 3), where a is the ratio of the response in the presence of antagonist to the response in its absence with the same hormone concentration. Using the derivation of Arunlakshana and Schild (15), one alternatively obtains from Eq. 1: $\log (DR - 1) = \log I - \log K_i$, or $\log (DR - 1) = \log I + pA_2$ (Eq. 4), where DR stands for the ratio of agonist concentra-

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tions giving the same activity E in the presence and in the absence of antagonist and $pA_2 = -\log K_i$.

Values indicated in the text refer to mean ± standard error of the mean. Statistical comparisons of pA_2 values were made by using a 3 × 3 analysis of variance (three-way for the three separate experimental groups and three-way for the three antagonists) according to the method of Mather (16).

Radioactive nucleotides were purchased from Amersham (Versailles, France) and histamine dihydrochloride from Merck (Darmstadt, Federal Republic of Germany). Cimetidine was kindly donated by Dr. M. E. Parsons (Smith Kline & French, Welwyn Garden City, United Kingdom), ranitidine by Dr. C. Alexandre (Laboratoires Glaxo, Paris, France), and ICI 125,211 by Dr. T. O. Yellin (ICI Americas Inc., Wilmington, Del.).

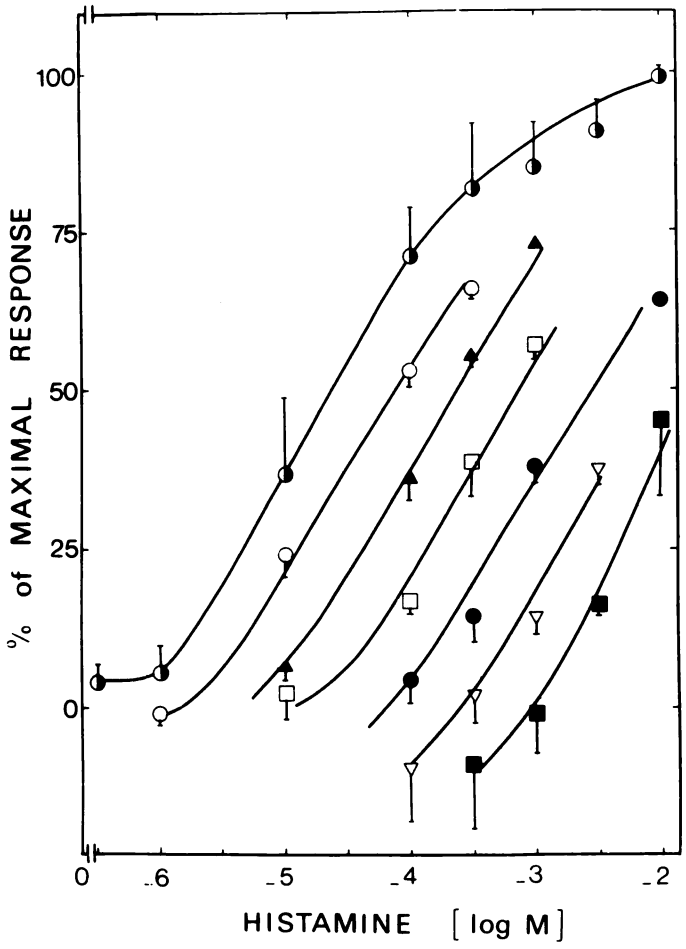


FIG. 1. *Effect of ranitidine on histamine-stimulated adenylate cyclase activity in guinea pig gastric mucosa cells*

Broken cells were incubated for 20 min at 30°, pH 7.8, in the presence of histamine alone (◑) or histamine and ranitidine at the following concentrations: 3×10^{-7} M (○); 10^{-6} M (▲); 3×10^{-6} M (□); 10^{-5} M (●); 3×10^{-5} M (▽); and 10^{-4} M (■). Adenylate cyclase activity was assayed as detailed under Materials and Methods, each determination being made in triplicate. Results are expressed as mean values ± standard error of the mean of mean triplicate determinations from three separate experiments (with the exception of two points corresponding to mean values of only two experiments and for which the standard error of the mean has not been indicated).

RESULTS

Adenylate cyclase activity of broken gastric cells was consistently stimulated by histamine over a range extending from a threshold concentration of 5×10^{-7} M to a maximal concentration of 10^{-2} M. Maximal stimulation corresponded to the production of 166 ± 59 pmoles of cyclic AMP per milligram of protein per minute (mean value ± standard error of the mean of three separate experiments), i.e., to 4.10 times the basal adenylate cyclase activity (40.5 ± 19.3 pmoles of cyclic AMP per milligram of protein per minute). Concentration-dependence curves were in good agreement with the Michaelis-Menten model detailed under Materials and Methods, and the concentration for half-maximal stimulation (K), estimated from the three experiments according to Eq. 2, was $1.55 \pm 0.29 \times 10^{-5}$ M. Histamine stimulation of adenylate cyclase activity was gradually and completely inhibited by increased concentrations of cimetidine, as well as of ranitidine, and of compound ICI 125,211. As is exemplified in Fig. 1 for ranitidine, all three H_2-receptor antagonists tested caused parallel shifts of histamine-stimulation curves to the right. For a fixed concentration of histamine (3×10^{-4} M), adenylate cyclase stimulation was progressively reduced to zero in the presence of increased concentrations of antagonists (Fig. 2). Representation of the data according to Eq. 3 resulted in three parallel lines with slopes not different from unity, thus suggesting simple competitive antagonism (not shown). From intercepts at the abscissa axis (i.e., half-maximal inhibition), K_i was estimated to $3.02 \pm 0.30 \times 10^{-7}$, 0.82

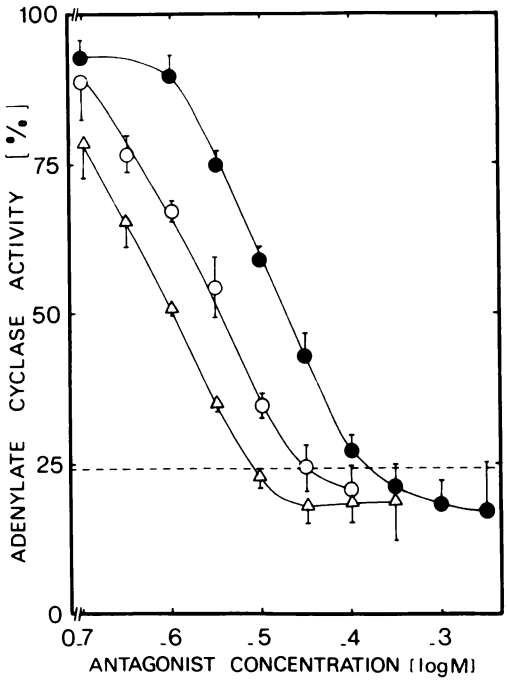


FIG. 2. *Effect of H_2-receptor antagonists on histamine-stimulated adenylate cyclase activity of guinea pig gastric mucosa cells*

Broken cells were incubated as detailed in the legend to Fig. 1 in the presence of 3×10^{-4} M histamine and without or with cimetidine (●), ranitidine (○), or ICI 125,211 (△) at increased concentrations. - - -, Basal activity. Mean values ± standard error of the mean of three separate experiments are shown.

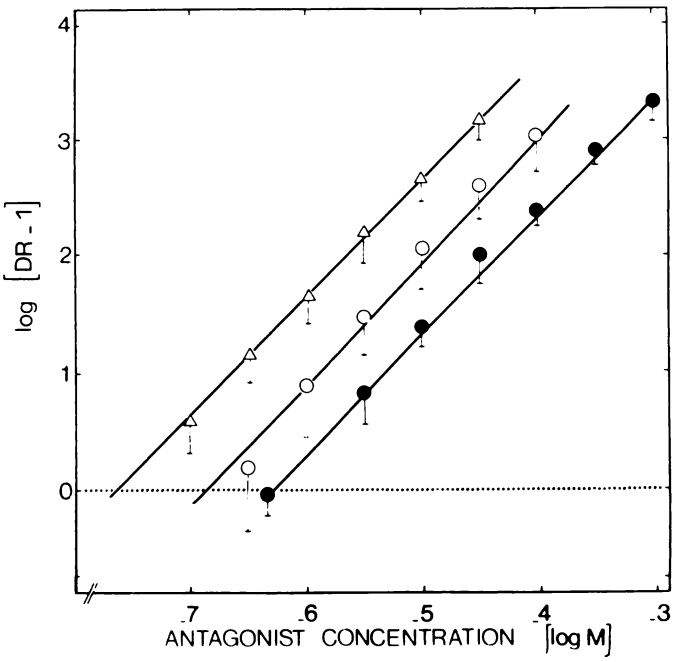


FIG. 3. *Schild representation of inhibition of histamine-stimulated adenylate cyclase in guinea pig gastric cells by cimetidine* (●), *ranitidine* (○), *and ICI 125,211* (△)

Mean values ± standard error of the mean of three separate experiments are shown.

± 0.08 × 10^{-7}, and 2.51 ± 0.75 × 10^{-8} M for cimetidine, ranitidine, and ICI 125,211, respectively (assuming that the exact K value corresponded to the mean value indicated above and that the histamine concentration in the medium corresponded to that actually present in the vicinity of the receptor).

The Schild plots established from the parallel displacements of the concentration-stimulation curves in the presence of increased concentrations of antagonists gave three apparently parallel straight lines (Fig. 3). The slopes of these lines as estimated from regression analysis were not significantly different from unity (Table 1). This finding is consistent with the observations reported above and strongly argues for a purely competitive mechanism of inhibition for all three antagonists. The pA_2 values were estimated from the intercepts at the zero ordinate in each experimental group (Table 1). These pA_2 values corresponded to apparent K_i values of 4.57 × 10^{-7}, 1.25 × 10^{-7}, and 2.39 × 10^{-8} M for cimetidine, ranitidine, and ICI 125,211, respectively. Statistical analysis (see Material and Methods) indicated that the effects of the three antagonists were significantly different at the error level $p < 0.01$ ($F_4^2 = 23.17$). Ranitidine was 4 times more potent than cimetidine at $p < 0.20$ ($t_4 = 1.667$), whereas ICI 125,211 was 5 times more potent than ranitidine at $p < 0.02$ ($t_4 = 3.813$) and more potent than cimetidine at $p < 0.01$ ($t_4 = 6.792$).

DISCUSSION

The values reported in this study for basal and histamine-stimulated adenylate cyclase activity in guinea pig gastric mucosa appear to be consistent with those found by previous authors on the same tissue. Anttila *et al.* (2) and Perrier and Griessen (1), respectively, reported mean basal activities of 48.5 ± 4.3 and 22.55 ± 1.4 pmoles of cyclic AMP per milligram of protein per minute and maximal stimulations corresponding to 5 and 3.2 times the basal activity. That higher standard errors are inherent to our study is likely to be the consequence of the smaller number of experimental groups. Indeed, considerable variations have been reported to occur from animal to animal. For instance, values ranging from 4.6–53.4 pmoles of cyclic AMP per milligram of protein per minute were observed by Perrier and Griessen for a group of 48 animals (1). Such variations could be accounted for by the presence of varied amounts of endogenous histamine in the preparations. This would be in accord with the inhibition of basal activity by the H_2-receptor antagonists that is evident in Figs. 1 and 2. On the other hand, experimental variations on triplicate determinations within each experimental group ranged, respectively, from 3 to 24% and from 0.5 to 4.4% for basal and histamine-stimulated activity in the presence or absence of antagonist. However, in this study the analysis of variance made it possible to take into consideration error linked to variations between experimental groups in the estimation of statistical significance.

From the quantitative analysis of the data, it is apparent that the effects of histamine and H_2-receptor antagonists are adequately described according to the classical Michaelis-Menten model for competitive agonist-antagonist interaction at a common receptor. The three antagonists are therefore suggested to have a similar competitive mechanism of action on biological functions mediated by this receptor *in vivo*.

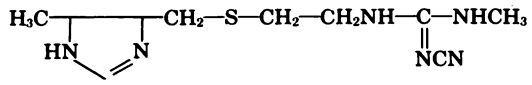
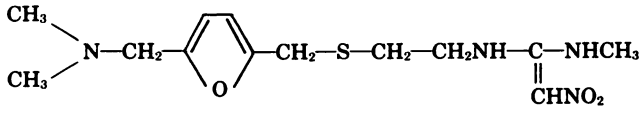
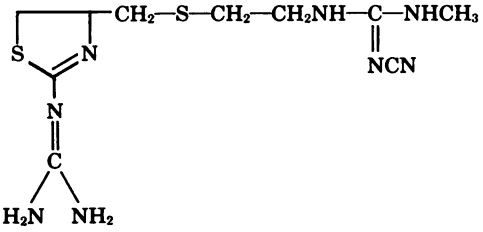
Estimations of K_i values and pA_2 values according to the method of Arunlakshana and Schild (15) from displacements of concentration-stimulation curves appear to be in good agreement with the estimations derived from inhibition curves with a fixed concentration of histamine. In all experiments, individual pA_2 values for ranitidine were higher than those for cimetidine, whereas individual pA_2 values for ICI 125,211 were higher than those for ranitidine. Statistical comparisons of the pA_2 values suggest the following hierarchy of potency: cimetidine ≤ ranitidine < ICI 125,211. This finding is consistent with the observations reported by others on guinea pig right atrium as a typical histamine H_2-receptor model. In this target, mean pA_2 values for cimetidine inhibition of positive inotropic action of histamine ranged from 6.40 to 6.68 (11, 12, 17, 18), and mean pA_2 values for ranitidine and ICI 125,211 were 7.20 (7.01 – 7.45) (11) and 7.8 (7.72 – 7.96) (12), respectively. These values are in excellent accord with those reported here and, in this respect, our study strongly supports the theory that histamine-stimulated adenylate cyclase activity in guinea pig gastric mucosa is specifically relevant to the histamine H_2 receptor.

The results are also consistent with the reported observations on histamine-stimulated gastric acid secretion *in vivo* in the rat (11), the dog (11, 12, 19), and man (20, 21). According to these observations, all three antagonists behave as competitive inhibitors, with relative potency being approximately as follows: cimetidine: 1 < ranitidine: 4 < ICI 125,211: 10.

TABLE 1

Structure-activity relationship of the three histamine H_2 antagonists studied

pA_2 values and slopes were calculated for each antagonist from three separate experiments similar to that shown in Fig. 1 for ranitidine.

Antagonist	Structure	pA_2	Slope	r
Cimetidine		6.34 ± 0.14	0.99 ± 0.38	0.998
Ranitidine		6.90 ± 0.33	1.07 ± 0.01	0.997
ICI 125,211		7.62 ± 0.27	1.02 ± 0.02	0.998

The above data strengthen the hypothesis that histamine stimulation of gastric acid secretion is mediated through an H_2-type receptor coupled to adenylate cyclase. Nevertheless, the present study cannot support the assertion that the effects on adenylate cyclase activity observed *in vitro* mimic exactly the effects on acid secretion *in vivo*. Discrepancies have been reported between the pA_2 values estimated for cimetidine inhibition of histamine-stimulated adenylate cyclase activity on uterus and atria and cimetidine inhibition of histamine-stimulated gastric acid secretion *in vitro*, but these have been suggested to result from overestimation of the antagonist concentration at the gastric receptor (22). On the other hand, effects on gastric adenylate cyclase were shown not to discriminate clearly between H_2- and H_1-receptor antagonists, although H_1-type antagonists have been proven to have no significant effect on acid secretion *in vivo* (1, 23). Thus, in gastric mucosa (5) as well as in brain (24, 25), high concentrations of H_1 antagonists were suggested to be competitive antagonists at the H_2 receptor. In this respect, however, we have proposed a model suggesting that H_1-antagonists interact *in vitro* (not *in vivo*) with the regulatory unit of the H_2 receptor (3).

Furthermore, important conclusions can be drawn with respect to the structural demand at the H_2 receptor. This has been suggested to involve two kinds of sites: (a) a functional site, anionic in nature, that interacts with the protonated ethylamine side chain of histamine, and (b) one (or more) binding site(s) that interact with the imidazole ring. In studies of large series of analogues, requirement of this imidazole ring in the molecular structure of H_2 antagonists has been postulated (22). In agreement with these current views, it was found that the three antagonists studied indeed had side chains which were nearly identical and resembled the ethylamine side chain of histamine, but with mofidications resulting in increased chain length and deprotonation of the NH_2 terminus (Table 1). However, at variance with previous suggestions, the ability of ranitidine and compound ICI 125,211 to compete with histamine for receptor occupancy clearly demonstrates that the imidazole ring is not required for the antagonists to be recognized by the receptor. Such a finding is consonant with the previous suggestions by Green and associates (24, 25) that tricyclic antidepressant drugs and hallucinogen α-lysergic acid diethylamide (which lack the imidazole ring) can block the histamine H_2 receptor in brain. Moreover, since the potencies of ranitidine and ICI 125,211 are higher than that of cimetidine, one may suggest that future research on the H_2-receptor antagonist should instead be directed toward molecules with nonimidazole structures.

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